# A Novel Gene Therapy For Rett Syndrome Through Reactivation Of The Silent X Chromosome

Samantha Powers, Song Lou, Salvatore Nardini, Shibi Likhite, J. Andrea Sierra Delgado, Megan Baird, Ottavio Vitolo, Florence Roussel, Abuzar Kaleem, Maura Schwartz, Shrestha Sinha Ray, Sanchita Bhatnagar, Kathrin Meyer

## Presented by Kathrin Meyer at ASGCT, May 18th, 2022, Washington, DC

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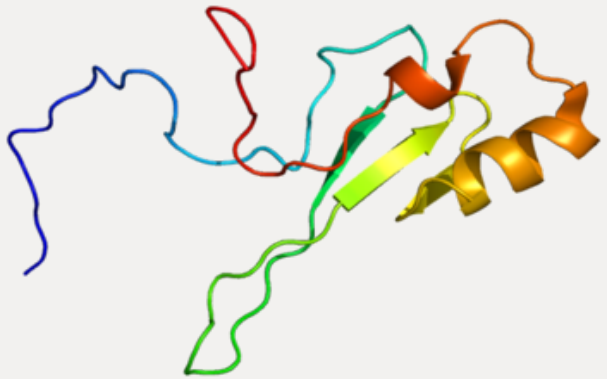
# Rett Syndrome

- **Devastating progressive neurodevelopmental disorder**
- **Affects approximately 1 in 10,000 girls**
- **Loss of developmental milestones around 6-18 months of age, followed by progressive loss of motor and cognitive function**
- **Current treatment limited to managing symptoms**

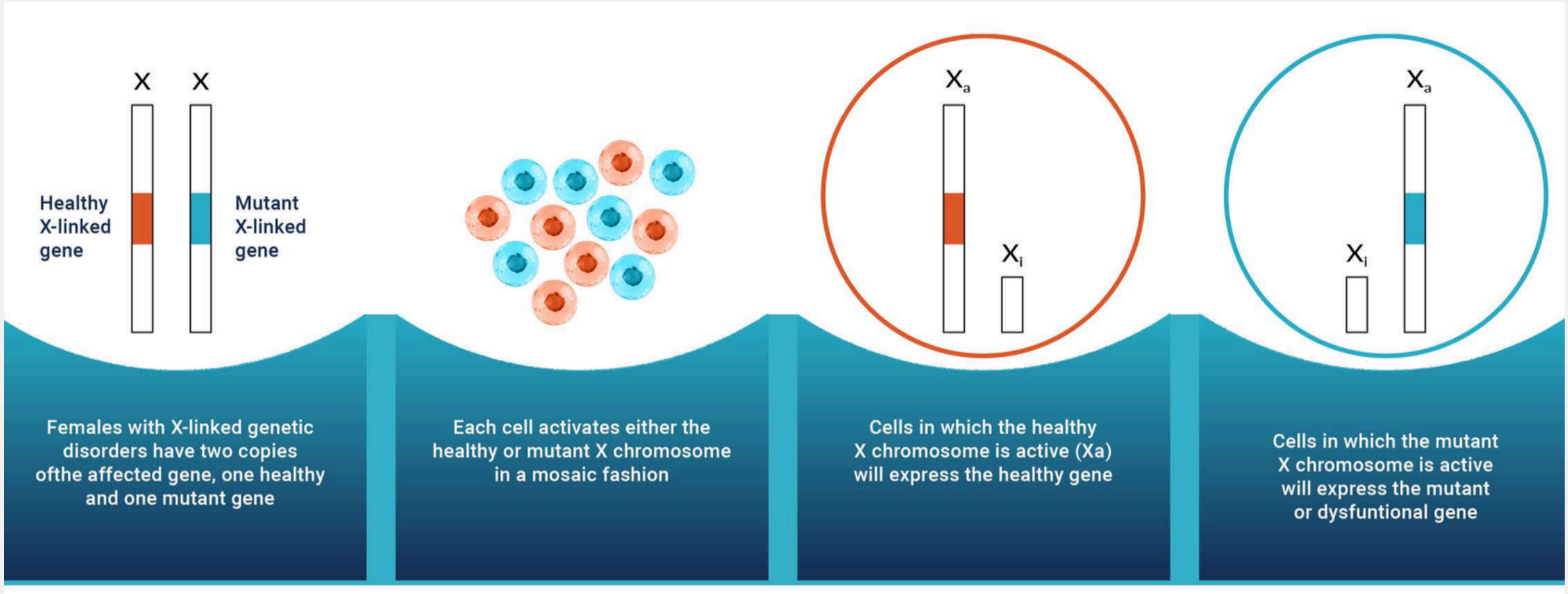
# Methyl CpG Binding Protein 2 (MeCP2)

- **Loss of function mutations in MeCP2 cause Rett Syndrome**
- **Ubiquitously expressed transcription factor**
- **Broadly regulates gene expression**
- **MeCP2 gene is located on X chromosome (Xq28)**
- **Gene Replacement strategies are difficult as overexpression is a major concern**

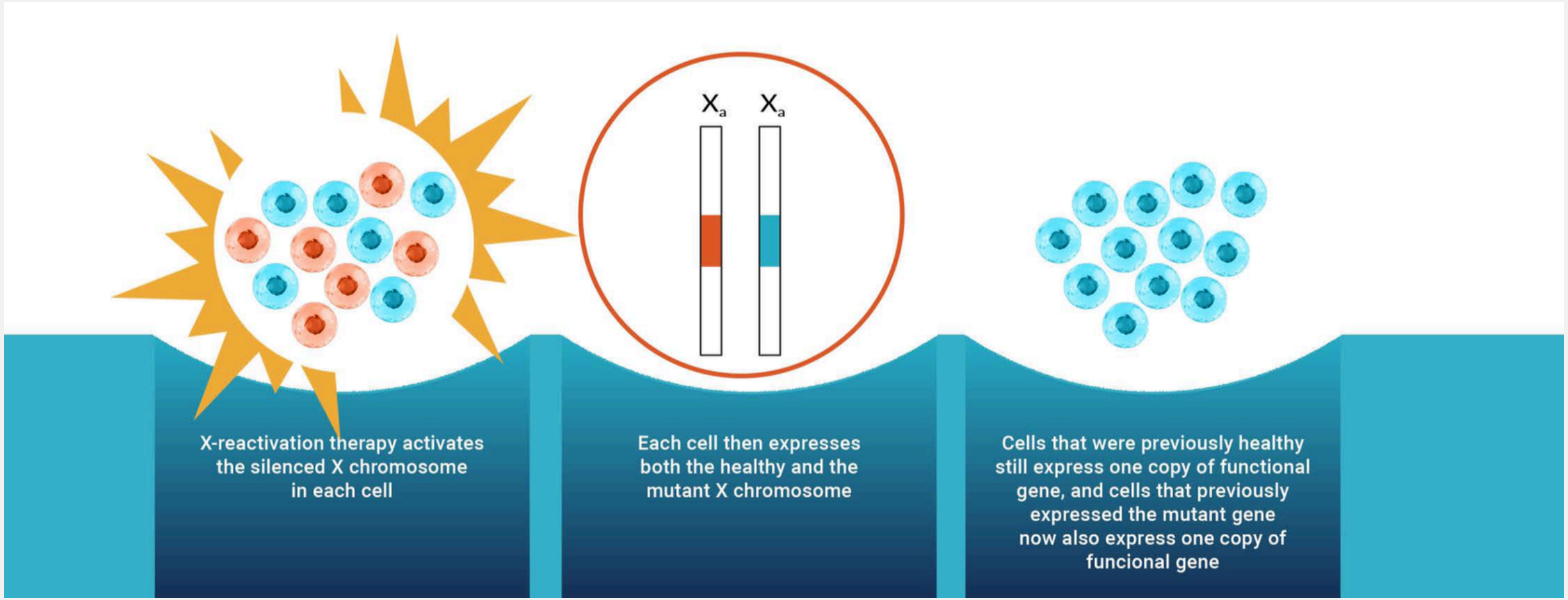


**MeCP2 Protein**

# X Reactivation as Therapeutic Strategy for Treatment of X-linked Disorders in Females



# X Reactivation as Therapeutic Strategy for Treatment of X-linked Disorders in Females

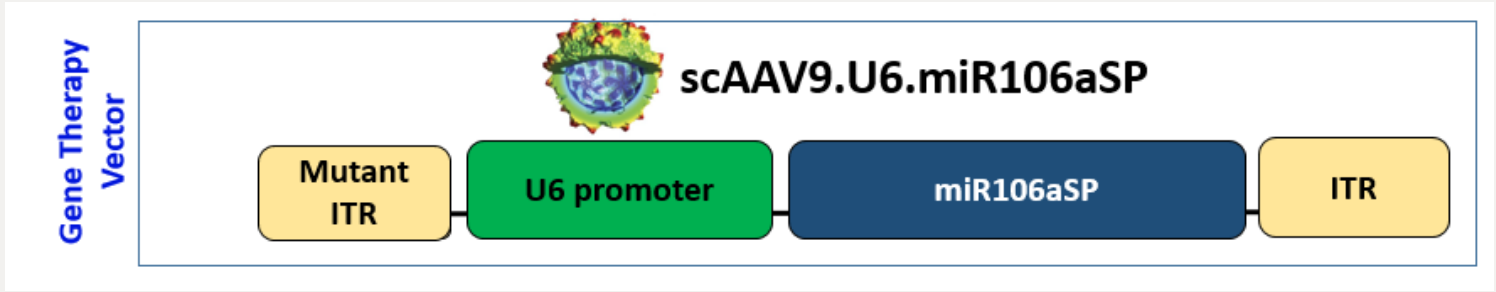


# X Reactivation as Therapeutic Strategy for Treatment of X-linked Disorders in Females

- **X inactivation less rigid than previously thought, many genes were shown to be expressed by both alleles in females**
- **Angelman Syndrome as POC that long non-coding RNA mediated silencing can be reversed**
- **10+ years studies in mechanisms of X inactivation**
- **Discovery of miR106a as major regulator of X inactivation identified through different screening methods**

**Sanchita Bhatnagar, PhD UVA – now UC Davis**

# AAV9.miR106aSP As Gene Therapy Approach for X Reactivation




- **miR106a knockout mice do not display any disease phenotype (Ventura et al, Cell 2008) and unpublished data from Meyer lab**
- **X reactivation allows expression of MeCP2 from the endogenous locus with all regulatory elements present**

# MeCP2 Transcription And Expression Regulation Is Diverse

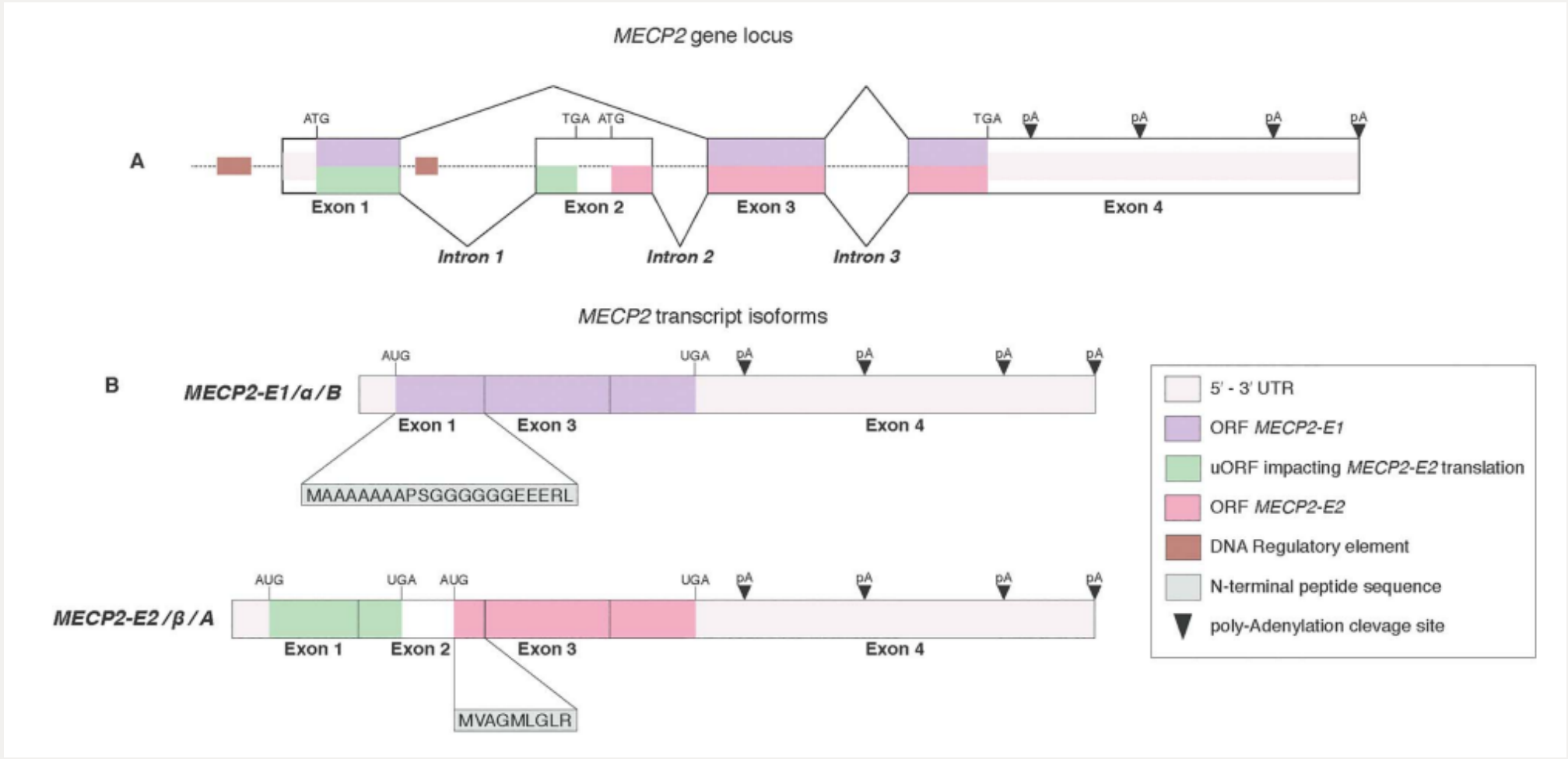



**Figure from Carvalho Rodrigues et al, Human Molecular Genetics, 2020**

# Predicted and Confirmed Protein and miR Binding Sites in MeCP2 3'UTR

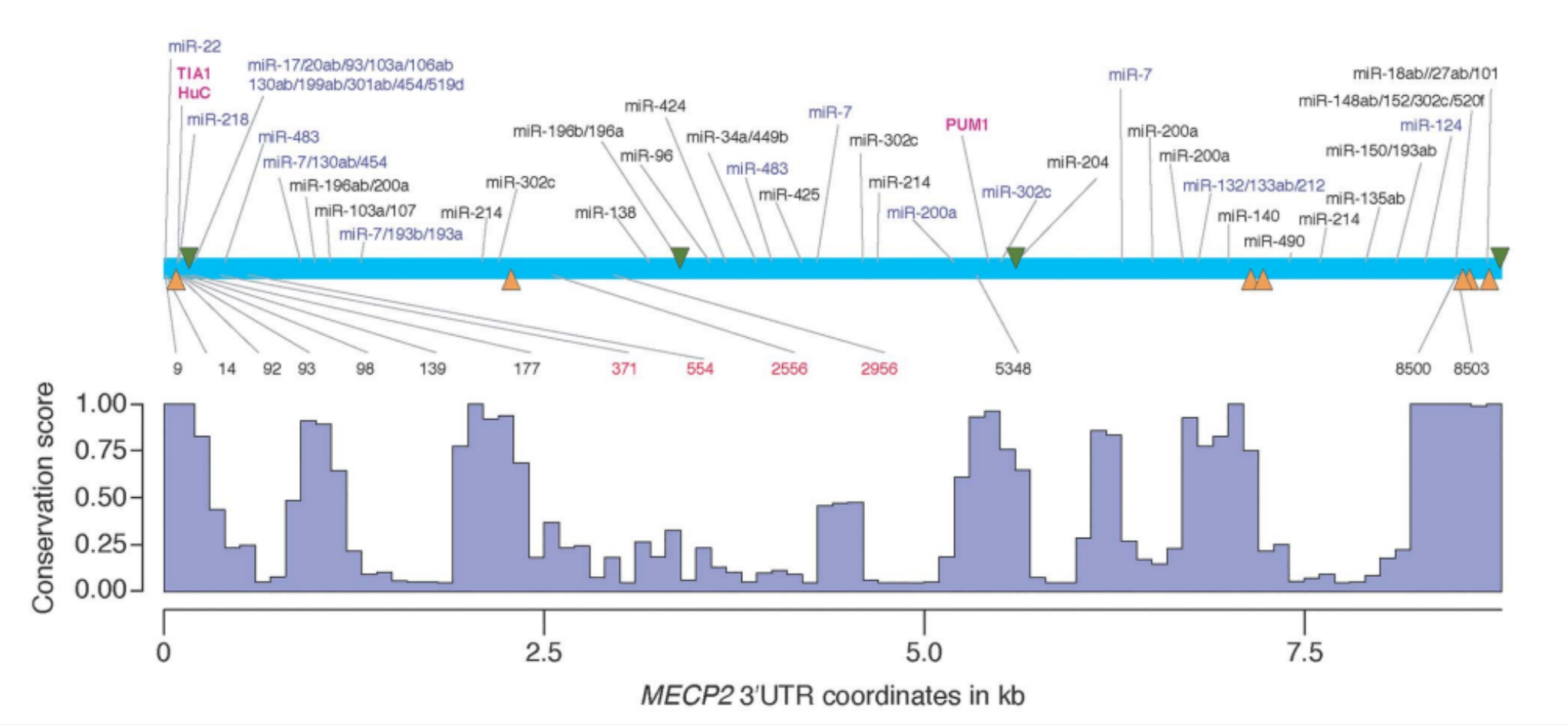
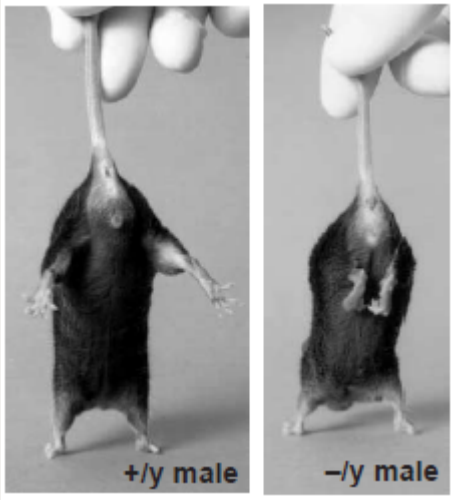



**Figure from Carvalho Rodrigues et al, Human Molecular Genetics, 2020**

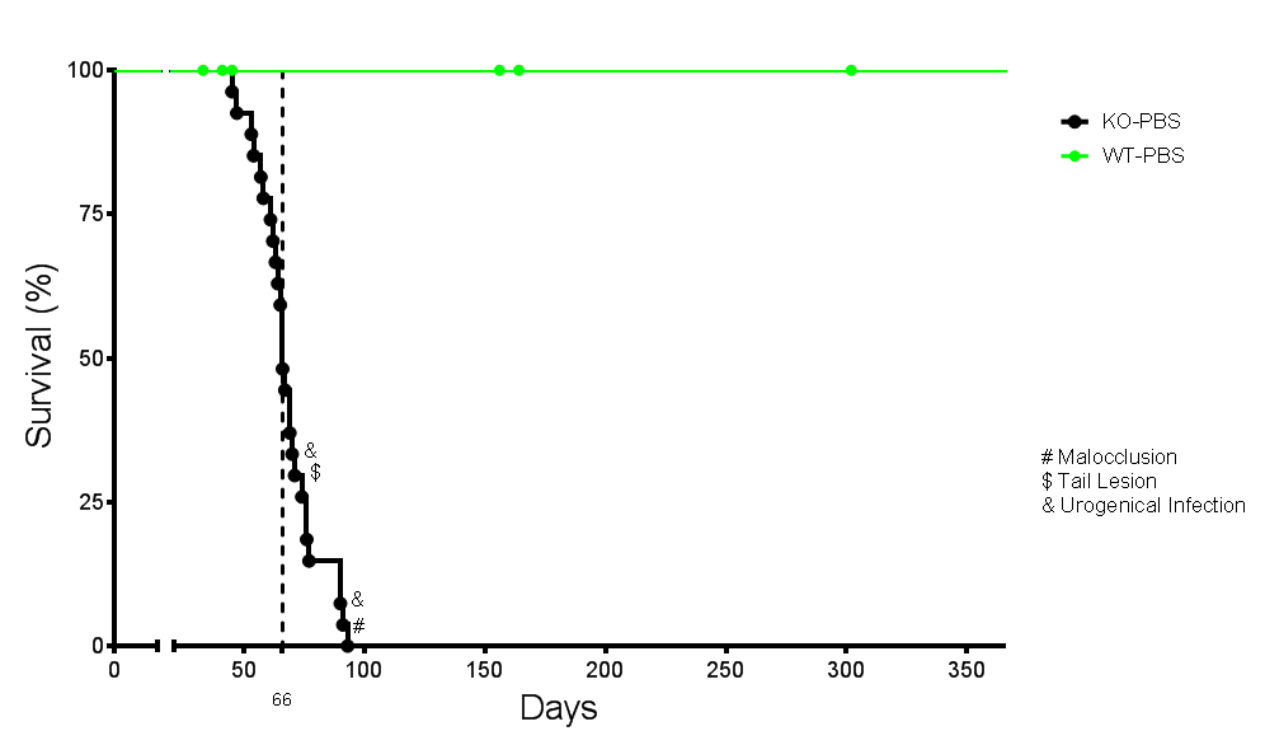
# Mouse Models Available for Rett Syndrome

- **Standard mouse model for studying Rett Syndrome: “Bird” Male KO mouse**
- **Males only have one X chromosome; all cells carry a deletion of MeCP2**

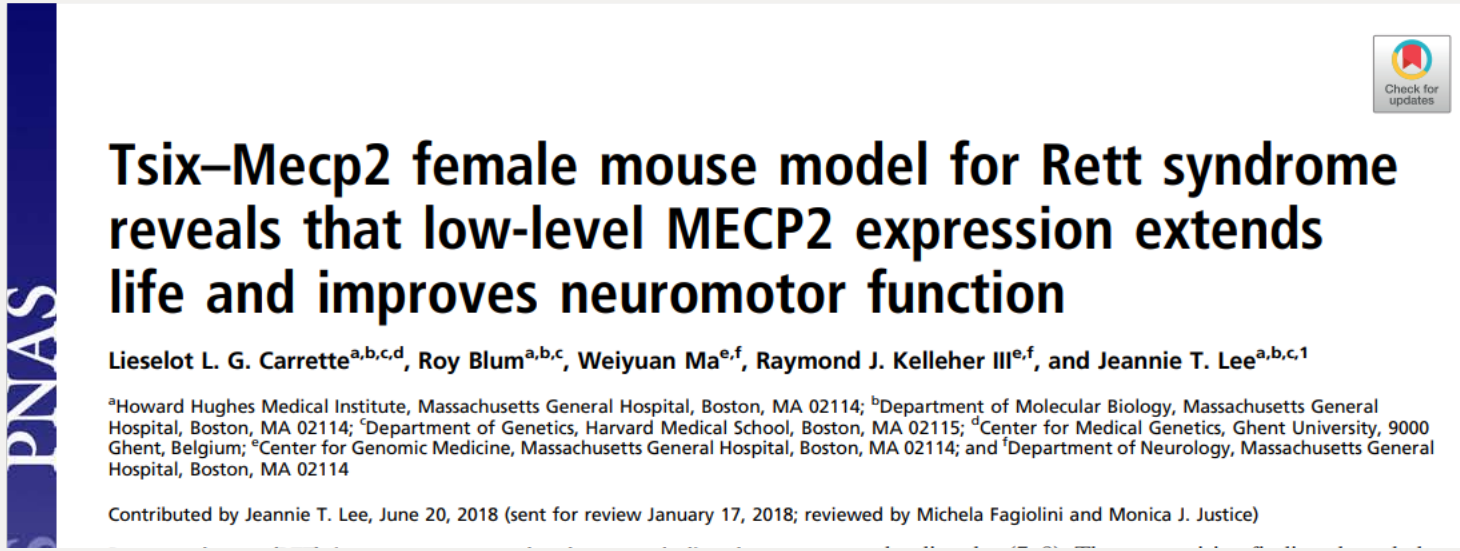



(Guy *et al*. 2007 Nature)

- **Severe, reduced survival, behavioral abnormalities**
- **Can’t be used to test X reactivation**



# Tsix-MeCP2 Mouse Model Represents the Worst-Case Scenario Female Patient



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## Tsix–Mecp2 female mouse model for Rett syndrome reveals that low-level MECP2 expression extends life and improves neuromotor function

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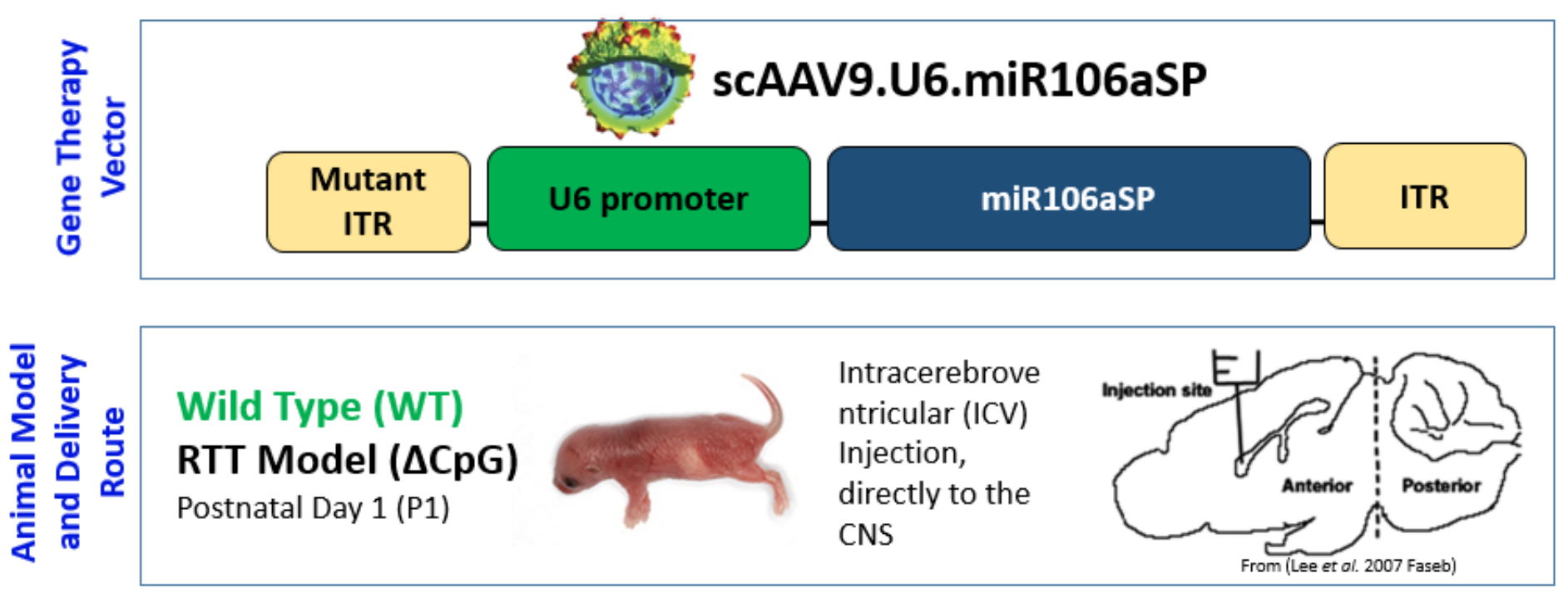
[a]Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA 02114; [b]Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114; [c]Department of Genetics, Harvard Medical School, Boston, MA 02115; [d]Center for Medical Genetics, Ghent University, 9000 Ghent, Belgium; [e]Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA 02114; and [f]Department of Neurology, Massachusetts General Hospital, Boston, MA 02114

Contributed by Jeannie T. Lee, June 20, 2018 (sent for review January 17, 2018; reviewed by Michela Fagiolini and Monica J. Justice)

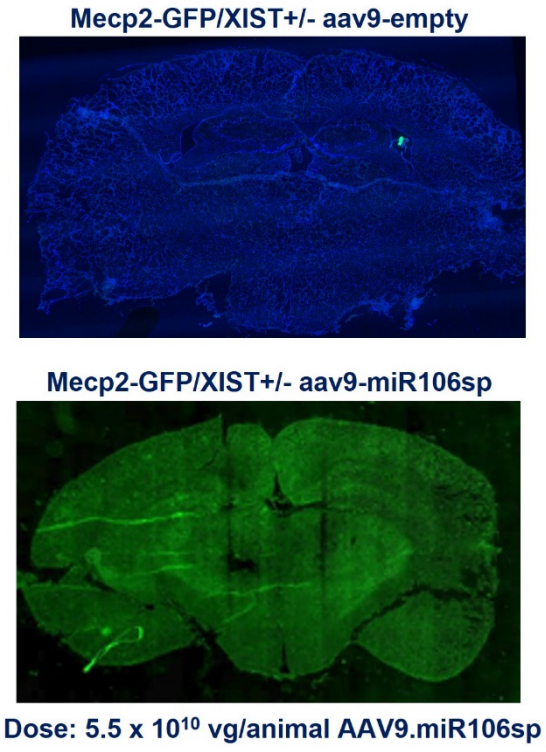
- **X Chromosome with MeCP2 KO active in all cells**
- **Healthy MeCP2 gene present on the silent X chromosome in all cells**
- **Genetic POC that X reactivation can impact MeCP2 loss of function disease phenotype**
- **Mouse model also available with MeCP2-GFP reporter gene on silent X chromosome**

# Study Design for Testing of Efficacy of scAAV9.miR106aSP



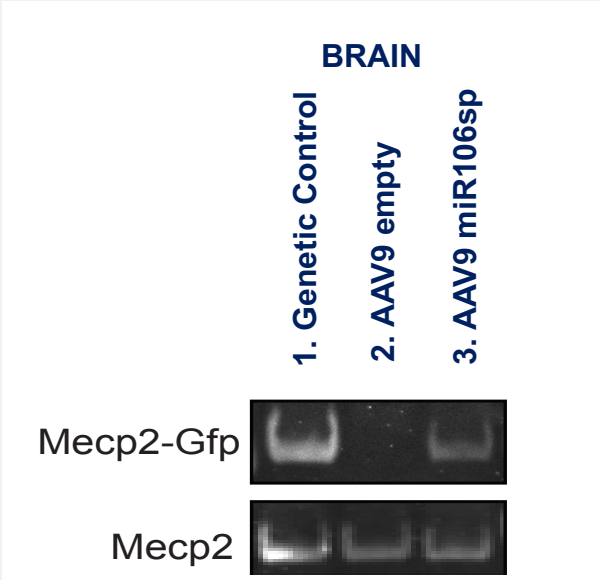

- **Postnatal day 1 or 2 injections to optimally mimic AAV9 transduction patterns seen in larger animal species**
- **Single-Dose POC efficacy study followed by dose-response (currently ongoing)**

# scAAV9.miR106aSP Allows Widespread X-Reactivation Throughout Mouse Brain

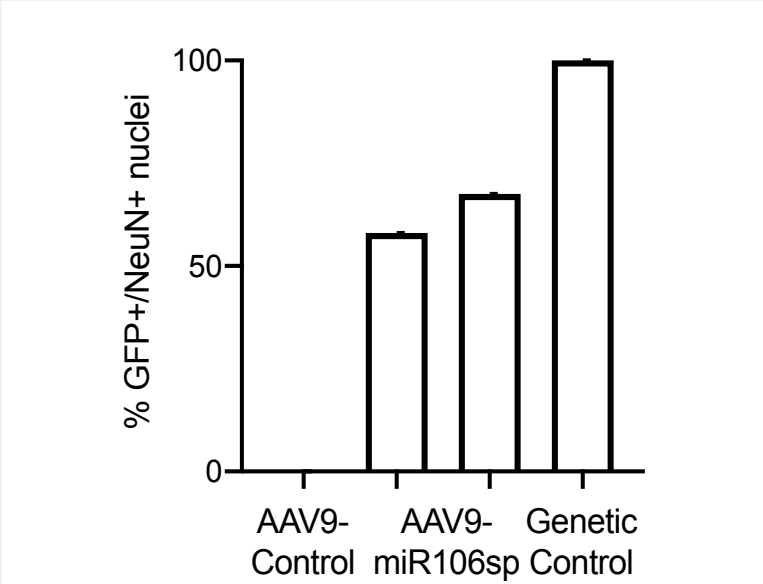



Brain cross section showing widespread re-activation of MeCP2-GFP expression throughout the entire brain

→ **Reporter Mouse carries MeCP2-GFP fusion gene on constitutively silent chromosome**
→ **MeCP2-GFP fusion protein is only expressed when silent X Chromosome gets activated**

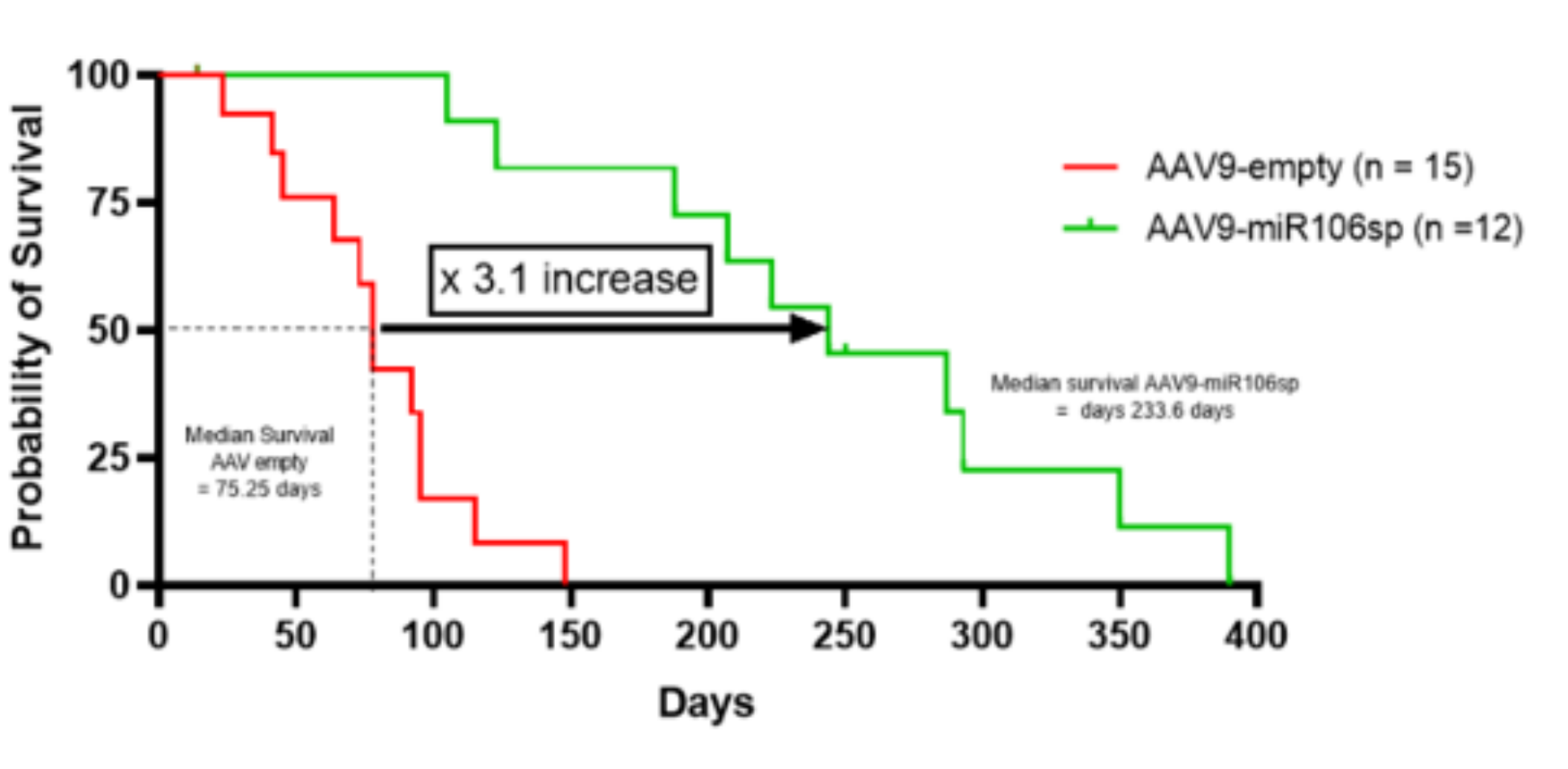



Western Blot confirms expression of MeCP2-GFP fusion protein in AAV9.miR106sp treated animals



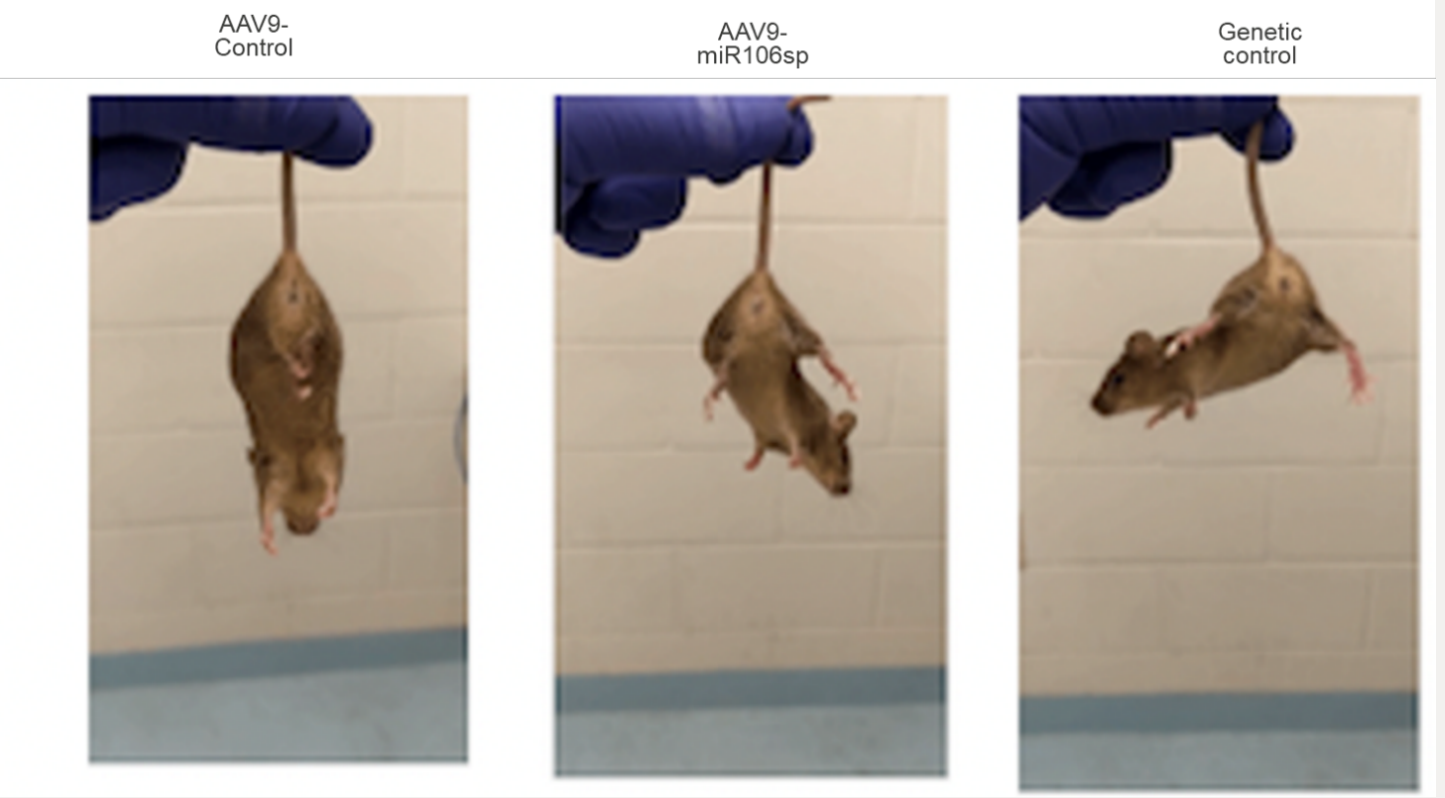

Fluorescent cell sorting from cortical suspensions show >50% of neurons reactivated the silenced X chromosome at 5.5 x $10^{10}$ vg/animal

# scAAV9.miR106aSP Significantly Increased Survival



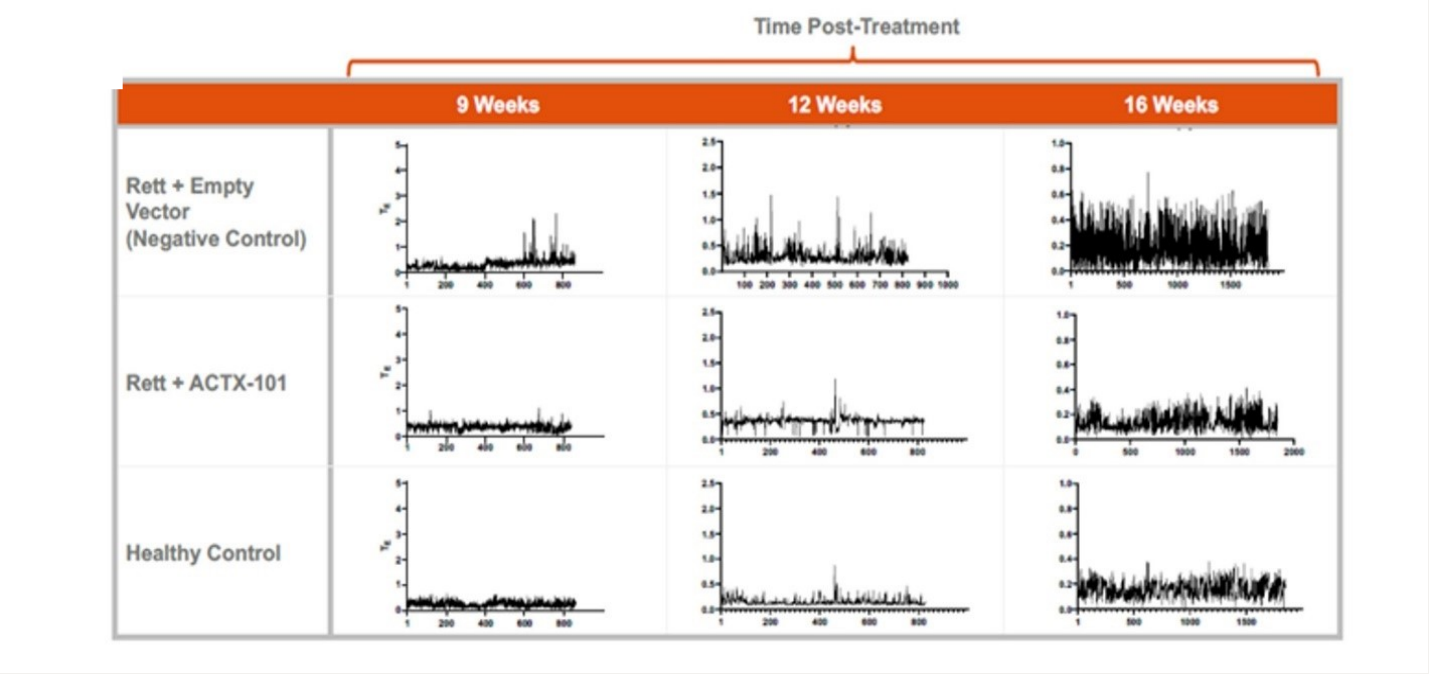

- **Median survival increased from 75 days to 234 days**

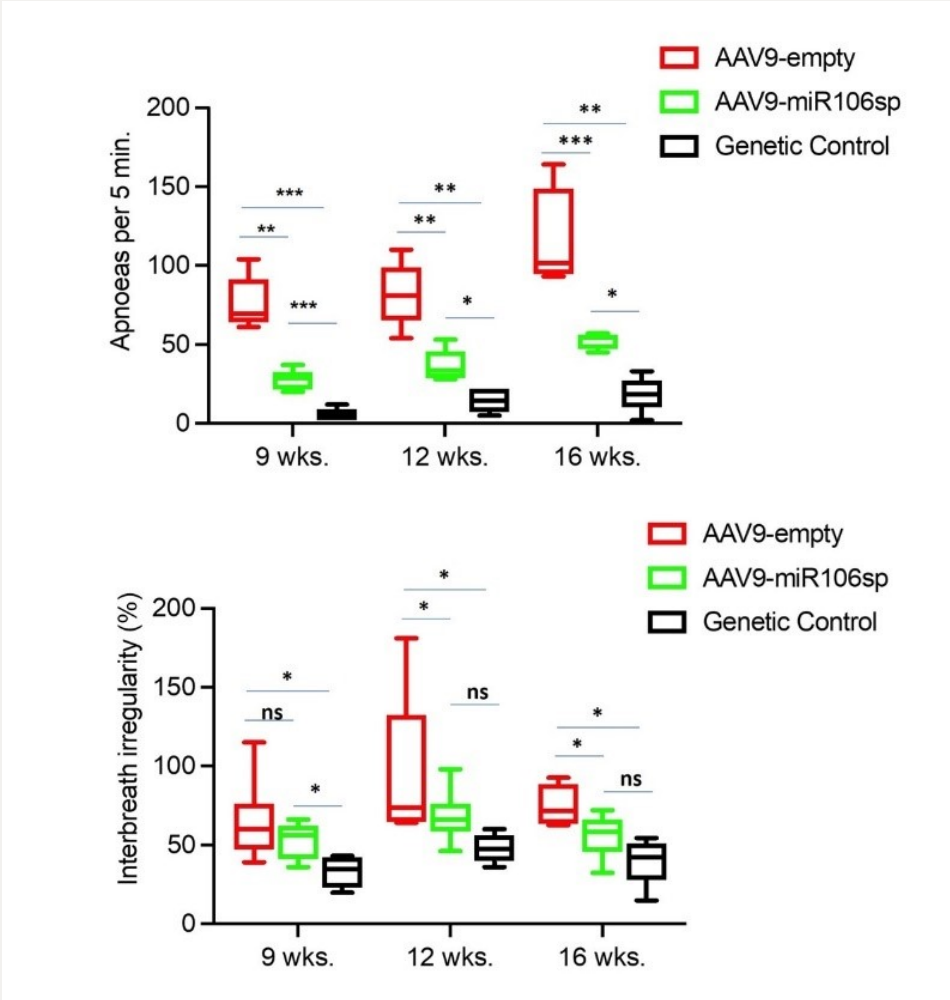
# scAAV9.miR106aSP Strongly Impacted Behavior and Physical Health



# scAAV9.miR106aSP Strongly Impacted Behavior and Physical Health

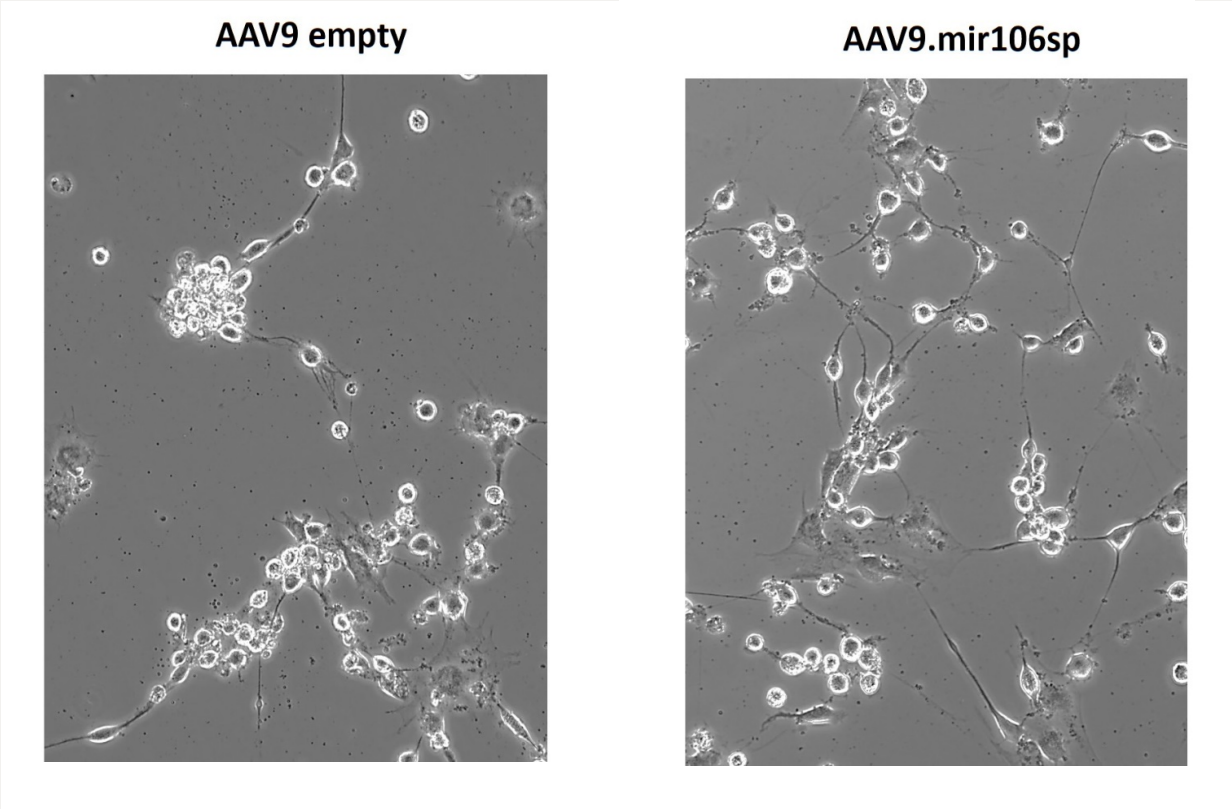
# scAAV9.miR106aSP Strongly Impacted Abnormal Breathing Patterns

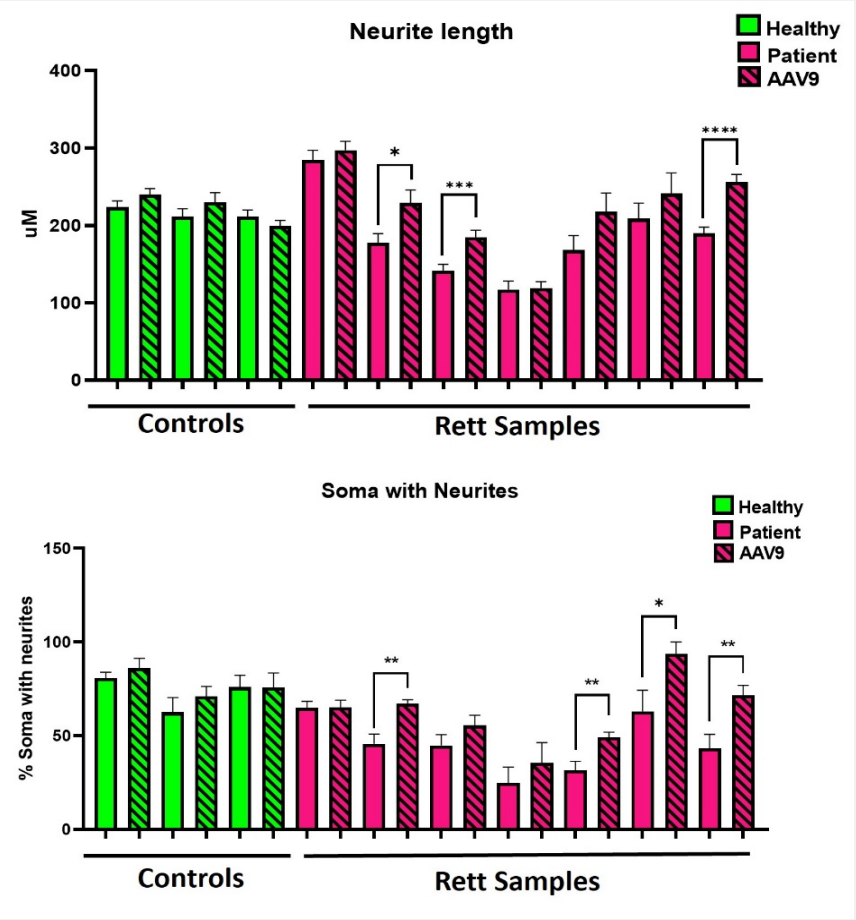



- **Both apneas and inter-breath irregularity significantly improved**

# scAAV9.miR106aSP Shows Beneficial Effects on Soma Size, Neurite Length/Number In Two Independent In Vitro Models




- **Rett patient neurons made from skin fibroblasts respond to miR106aSP treatment**
- **Rett iPSC derived neurons show MeCP2 re-expression, increased soma size, increased branching and normalized electrophysiological properties**

# scAAV9.miR106aSP Treatment Is Safe And Well Tolerated In Mice And Non-human Primates At Wide Dose-Range Up To 6 Months Post Injection

| Dose Response Expression / Safety Study in Wild Type Mice | Pilot Safety Study in Wild Type Non-Human Primates |
|---|---|
| • 3 doses under evaluation:<br>  • $2 \times 10^{10}$ vg/animal<br>  • $6 \times 10^{10}$ vg/animal<br>  • $1.8 \times 10^{11}$ vg/animal<br>• Over 30 female animals per dose enrolled<br>• Animals sacrificed at 1-, 3- and 6-months post injection<br><br>**No difference observed in weights, phenotype and behavior up to 6 months** | • Two 4-year-old Rhesus Macaque female NHPs<br>  • $6 \times 10^{13}$ vg/animal<br>  • Lumbar puncture CSF delivery<br>  • Followed by Trendelenburg tilting<br>  • 4 months in life<br><br>**No clinical or pathology findings**<br>**Full GLP Tox Study pending** |

# Acknowledgements


Nationwide Children's — When your child needs a hospital, everything matters.℠

Alcyone Therapeutics — Pioneering Next-Generation CNS Genetic Therapies

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Song Lou, PhD
Jeannie Lee, MD, PhD

***Sharing of the mouse model!***

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# Excellence in Research Award and a Meritorious Abstract Travel Award

**Samantha Powers, PhD**

# Thank You & Questions

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